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Antioxidant activities of Hydro-ethanol and Saponin extracts of *Terminalia schimeperiana* root

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ABSTRACT

In this present paper we have investigated the Antioxidant activity of Hydro-ethanol and Saponin fractions of Terminalia schimperiana root for its free radical scavenging activity by adopting ABTS and DPPH in vitro methods. The extracts were investigated for the antioxidant activity using 2, 2 - diphenyl, 1- picryl hydrazyl (DPPH) and 2, 2 azinobis-3-ethylbenzothiazoline-6-sulfonic acid (ABTS) scavenging activity, reducing capacity and competition with DMSO. The result suggested that the polar Hydro-ethanol fraction was found to have potent DPPH antioxidant activity with IC50 value of 19.36± 0.436 µg/ml and ABTS scavenging activity with IC50 value of 0.9420± 0.011 µg/ml, while the Saponin fraction has moderate DPPH scavenging activity with IC50 of 59.33± 0.417 µg/ml and moderate ABTS scavenging activity with IC50 value of 2.273± 0.036 µg/ml (Rutin DPPH IC50 value= 14.5±0.29 µg/ml, Rutin ABTS IC5O value= 0.2976± 0.012 µg/ml, Ascorbic acid ABTS IC50 value= 2.62± 0.20, Ascorbic acid DPPH IC50 value=9.51± 0.22).

Keywords: *Antioxidant, free radicals, Terminalia schimperiana, DPPH, ABTS*

INTRODUCTION

Many herbal plants play important role as antioxidant by providing protection to human against infection and degenerative diseases, thus inhibiting and scavenging free radicals such as superoxide, peroxyl, hydroxyl and oxygen radicals. This is possible because they contain compounds that neutralize free radicals and their action. Commonly, reactive oxygen species (ROS) such as

hydrogen peroxide (H_2O_2), hydroxyl radical and free radicals affect biological tissues due to oxidative stress (1). Studies have proved that transition metals such as the cations of iron and copper have the ability to catalyze the formation of ROS like hydroxyl radicals (2) which is known as Haber-Weiss, and Fenton-type reactions (3). Recently research has been directed towards "Natural antioxidants" from the herbal plants due to safe therapeutic index. In Nigeria indigenous herbal plant remedies have been used in treatment of various diseases such as diabetes, cancer, sexual dysfunction etc. Example of such plant is *Terminalia schimperiana* (Idi; Yoruba). It is a plant that belongs to the order myrtales and family combretaceae, a broadleaved small tree that can reach up to 7–14 m, variably deciduous in the dry season to semi-evergreen, depending on the climate. The leaves are alternate, simple, and elliptic to obovate, 9–15 cm long and 3–8 cm broad, green above with pale undersides, the flowers are tiny and form pale spikes at the base of the leaves, the fruit is a samara with a single wing 6–9 cm long, that turns brown with age. It can be found in open forest habitats with more than 1300 mm of rainfall per year, when it is found in closed forest, it typically part of the forest canopy and it may be the dominant tree species where it is found (4). The objectives of the present study are to extract hydro-ethanol fraction, Saponin fraction, and to determine the antioxidant activity of the fractions using 2, 2 - diphenyl, 1- picryl hydrazyl (DPPH) and 2, 2 azinobis-3-ethylbenzothiazoline-6-sulfonic acid (ABTS) scavenging activity, reducing capacity and competition with standard antioxidant such a Rutin and Ascorbic acid.

MATERIALS AND METHODS

Plant material

The root of *Terminalia schimperiana* was collected from Oko, a village from Irepodun local government area of Kwara state Nigeria. Identification and authentication of plant was carried out at the botany unit of the Department of Pure and Applied Biology, Ladoke Akintola College of science and technology, Ogbomoso, Nigeria.

Hydro-ethanol extract preparation

The root was cleaned, cut into pieces and shade dried, then the dried pieces were pulverized into course powder using an electric grinder. 130g of the course powder was subjected to maceration in 700ml of 70% (v/v) hydro-ethanol with constant shaking for 120 hrs at room temperature. The extract was filtered with Marcelin cloth and the filtrate was further filtered with Whatman no. 1 filter paper. The filtrate was concentrated using rotatory vacuum evaporator till all the ethanol has been removed and the remaining extract was concentrated on water bath after which the percentage yield was calculated and preliminary phytochemical tests were carried out on the extract.

Saponin extraction

5gms of mother extract was reluxed with 90%v/v methanol (25ml) for half an hour. The residue was extracted two more times by 25ml methanol. The methanolic extract was distilled off the solvent. The soft extract left after distillation of alcohol was treated with petroleum ether 60-80ºC, 25ml by refluxing for half an hour. It was cooled and the solvent removed by decantation.

The same soft extract was treated successively with 25 ml of chloroform and 25 ml of ethyl acetate

the soft extract was dissolved (after three extractions cited above) in 25ml of 90%v/v methanol, was filtered and was concentrated to 5ml. the above concentrate was then added drop by drop with constant stirring to 25ml acetone in order to precipitate the saponins. The precipitates were filtered, collected and dried to a constant weight at 105ºC. The dried extracts thus obtained were used for the assessment of antioxidant activity through DPPH and ABTS *in vitro* models. Preliminary qualitative and qualitative analysis was carried out to ascertain the presence of phytochemicals present.

Antioxidant assay

DPPH radical scavenging assay

The antioxidant scavenging ability of the extract towards the stable 2, 2 - diphenyl, 1- picryl hydrazyl (DPPH) free radical was determined using the methods described by Gudda et al., 2004 and Sanchez-Moreno, 2002. (5), (6). The assay was carried out in a 96 well microtiter plate, incubated at 37^0C for 20 minutes and the absorbance of each well measured at 490nm, Using ELISA reader against the corresponding test and standard blanks and the remaining DPPH calculated. IC_{50} (inhibitory concentration) i.e. concentration of the extract required to scavenge 50% of free radical DPPH was calculated based on control reading by following equation.

$$\% \text{ of Inhibition} = \frac{A_{con} - A_{test}}{A_{con}} \times 100$$

A_{con} - is the absorbance of the control reaction

A_{test} - is the absorbance in the presence of the sample of the extracts.

ABTS radical scavenging assay

Using the method described by Roberta et al., 1999 and Sanchez-Moreno, 2002, (7), (6) the antioxidant activity of the extract is determined by the reduction of the pre-formed radical monocation of 2, 2-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid (ABTS) which is generated by oxidation of ABTS with potassium per sulfate (a blue chromogen). Absorbance was measured

spectrophotometrically, after 20 mins at 734nm using ELISA reader and the IC_{50} value obtained is the concentration of the sample required to inhibit 50 % ABTS radical mono cation.

$$\% \text{ of Inhibition} = \frac{A_{con} - A_{test}}{A_{con}} \times 100$$

A_{con} - is the absorbance of the control reaction

A_{test} - is the absorbance in the presence of the sample of the extracts.

RESULTS AND DISCUSSION

Table 1: Showing the yield of *Terminalia schimperiana root* extract obtained

Sl. No.	Name of solvent	Colour	Consistency	Yield (%w/w)
1.	Hydro-Ethanol	Dark brown	Sticky	10.62
2.	N- Butanol	brown	Sticky	21.30

Table 2: The Qualitative Phytochemical analysis of Hydro-ethanol and Saponin fractions of *Terminalia schimperiana root*

Extract	Alkaloid	Glycoside	Flavonoids	Tannins	Saponin	triterpenes	Phenol
Hydro-Ethanol	+	_	+	+	+	+	+
Saponin	_	+	_	+	+	+	-

Table 3: Terminalia schimperiana ABTS and DPPH antioxidant values

Plant Extract	DPPH	ABTS
Hydro- ethanol	19.36± 0.43	0.94± 0.01
Saponin Extract	59.33± 0.41	2.27± 0.03
Standard		
tin	14.5±0.29	0.29± 0.01
Ascorbic acid	9.51± 0.22	2.62± 0.20
Note: (*IC50 values ± SEM µg/ml) *IC50 (Inhibitory Concentration) is the concentration of sample required to scavenge 50% of DPPH free radicals.		

DPPH Radical Scavenging Activity

The *in vitro* antioxidant activity of both hydro-ethanol and Saponin extracts were investigated using DPPH radical scavenging assay. This method evaluates the antioxidant activity based on the scavenging of stable DPPH radicals, since DPPH is a compound that is composed of a nitrogen-free radical which is easily quenched by a free radical scavenger (3), the scavenging reaction between DPPH and antioxidant compound (H-A) is due to the ability of antioxidants to change DPPH as a stable free radical to the DPPH-H (non- radical form). The degree of colour change by extracts or antioxidant compounds indicates their scavenging ability in terms of hydrogen donating ability (8). In table 3, it was observed that the polar Hydro-ethanol fraction was found to have highest potent DPPH antioxidant activity with IC_{50} value of 19.36± 0.43 µg/ml, Saponin fraction has DPPH scavenging activity with IC_{50} of 59.33± 0.41 µg/ml while the rutin DPPH antioxidant activity IC_{50} value is 14.5±0.29 µg/ml and Ascorbic acid antioxidant activity IC_{50} value is 9.51± 0.22. From the result, the hydro-ethanol extract of *Terminalia schimperiana* root inhibited higher DPPH free radical scavenging activity better than the Saponin extract at the same concentration and compares favorably with the standard rutin and Ascorbic acid DPPH antioxidant activities. The DPPH scavenging activity of the Saponin extract is very low when compared to the standard rutin DPPH activity yet it could still act as a free radical scavenger because it possess proton-donating potential. This finding is supported by the study of Mehdi Farshad et al., 2013 (9), who stated that the IC_{50} of crude extract of *Chlorophytum borivilianum* was less than that of total Saponin or indicated 2.5-fold stronger antioxidant activity than that of total Saponin.

ABTS radical scavenging activity

Among the two extracts, hydro-ethanol extract showed potent antioxidant activity with IC_{50} value of 0.94± 0.01 µg/ml and compared favorably with the standard rutin and Ascorbic acid antioxidant activity with IC_{5O} value of 0.29± 0.03 and 2.62± 0.20

respectively, followed by Saponin extract which showed a moderate scavenging activity with IC_{50} value of 2.27± 0.03 µg/ml when compared with the standard rutin and Ascorbic acid. The results of this study is supported by the findings of Ji Hye Lee et al., 2011 (10) who stated that phenolic content present in mung beans have a greater quality to eliminate free radicals than Saponin content and that the radical scavenging activities of Saponin were only marginal.

CONCLUSION

Antioxidant activity of *Terminalia schimperiana* root plant extracts were analyzed using free radical scavenging activity DPPH and ABTS, the two extracts shows free radical scavenging activity but the hydro-ethanol extract exhibited more free radical 2, 2–diphenyl-1- picryl hydrazyl scavenging ability than the Saponin extract and could be useful in preventing various diseases caused by free radicals.

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